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Determination of the Inclusion Geometry for the β-Cyclodextrin/Benzoic Acid Complex by NMR and Molecular Modeling

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Introduction

Cyclodextrins (CDs),[1] macrocycles formed by α(1−4) linked units of α-D-glucopyranose, are among the most widely used host molecules due to their solubility in water, and their torus creates a cavity with relatively low polarity in which organic molecules can be accommodated.[2] They have also been used as enzyme models.[3−6]

Any reliable analysis of a possible inclusion complex should cover three aspects: proof of actual inclusion, its stoichiometry, and stability (association constant). Moreover, a 3D structure for the supermolecule is also desirable. Among the techniques available, NMR spectroscopy is the most reliable. Studies on the two inner (H_3 and H_5) hydrogen atoms of CDs in the presence of a guest molecule provide valuable experimental data, although the fast equilibrium between complexed and free species prevents the straightforward determination of the 3D structure for the complex. Molecular modeling may offer insights on this aspect.[7−14]

Here we study the inclusion of a guest molecule (namely benzoic acid) into a host (β-CD) by combining experimental data obtained from NMR techniques and computational data from molecular mechanics calculations.

Benzoic acid has been proved to complex well with α-CD by different techniques;[15] nevertheless its complexation with β-CD has not been well established in literature[16] although it has been previously studied.[17]

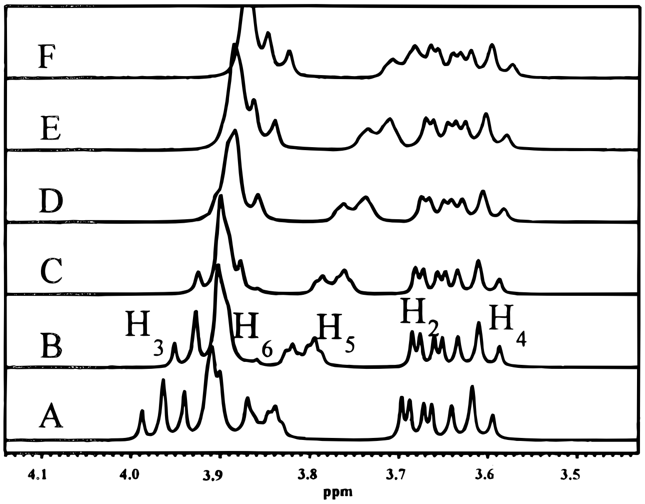
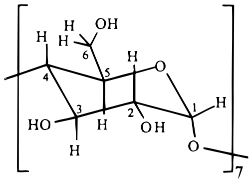
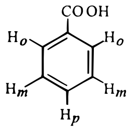


Figure 1. Partial 400 MHz ^1H NMR spectra (only host protons are shown) for solutions of β-CD/benzoic acid complexes. $[Host]_i/[guest]_i$ ratios: (A) 3.5; (B) 1.9; (C) 1.1; (D) 0.8; (E) 0.5; (F) 0.2.



β-CD



Benzoic acid

Results

NMR Results. The NMR spectra were recorded for six samples with host/guest ratios from 3.5 to 0.2. Each sample spectrum (Figure 1) denoted induced chemical shifts ($\Delta\delta_i$) for the inner protons of the host (H_3 and H_5) as well as for most of the guest protons (H_o and H_m). The largest displacements were observed for the inner β-CD protons. The formation of an inclusion complex in solution can be deduced from these data. Information on the stoichiometry and association constant can be extracted through treatment of the data from such simple experiments through the variation of the chemical shifts for different protons.

The β-CD anomeric proton (H_1) was used as internal reference throughout this work. Use of this proton as reference is justified by the following reasons: (a) the water signal is too wide, (b) H_3 and H_5 signal suffer shifts when complexing, (c) H_2 and H_4, although they are

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Table 1. Experimental $\Delta\delta_i$ Values (in ppm) for All the Studied Protons. Values are Relative to the Spectrum with a Smaller Concentration (mol/L) of the Species in the Study

sample	[β-CD]	[benzoic acid]	$\Delta\delta_i$ H_o	$\Delta\delta_i$ H_m	$\Delta\delta_i$ H_p	$\Delta\delta_i$ H_3	$\Delta\delta_i$ H_5
1	1.304×10^{-2}	0.3771×10^{-2}	−0.0429	0.0188	–	0.0000	0.0000
2	1.0595×10^{-2}	0.5679×10^{-2}	−0.0342	0.0100	−0.0086	−0.0263	−0.0332
3	0.8965×10^{-2}	0.7828×10^{-2}	−0.0240	0.0066	−0.0072	−0.0484	−0.0635
4	0.7335×10^{-2}	0.8608×10^{-2}	−0.0191	0.0051	−0.0057	−0.0616	−0.0812
5	0.5705×10^{-2}	1.0950×10^{-2}	−0.0079	0.0046	−0.0018	−0.0748	−0.1027
6	0.2445×10^{-2}	1.1660×10^{-2}	0.0000	0.0000	0.0000	−0.0829	−0.1230

Table 2. Relative NOE Values Observed on Presaturation of Different Protons of the β-CD/Benzoic Acid Inclusion Complex Using the 1D-ROESY Technique. Negative and Positive Signs Indicate the Absence or the Nonintegrable Presence of Enhancements, Respectively

	presaturated				
observed	H_o	H_p	H_m	$H_{3(6)}$[a]	H_5
H_o		(−)	1	0.12	1
H_p	(−)		(−)	(−)	(−)
H_m	1	(+)		0.08	0.18
H_3	0.25	(−)	0.29		
H_5	0.24	(−)	0.16	1	

[a] Overlapping signals for H_3 and $H_{6/6}$!

outside the cavity, they can be affected by the complexation since they are near the rims, (d) its location ensures the smallest (if any) induced shift. Table 1 contains the experimentally determined $\Delta\delta_i$ values (in ppm) and the host and guest concentrations. Guest concentrations used on Table 1 were experimentally determined from direct integration of the NMR signals. Job's method[18] furnished a 1:1 final stoichiometry for the complex. An association constant of 48.5 M^{-1} was computed using the experimental $\Delta\delta_i$ together with the corresponding $[host]_i$ and $[guest]_i$ (concentrations at each sample, see Table 1) using a computer program.[19]

1D-ROESY experiments[20] were carried out on the sample having a host/guest ratio of 0.8 to ensure detectable guest signals. Table 2 shows the relative quantification of the observed NOE enhancements. Large intramolecular NOE values were observed as expected. Intermolecular NOE values are relatively small but significant. The absence of NOE on the CD protons on saturation of H_p is relevant, as well as the presence of intermolecular NOE on saturation of H_o, H_m, H_3, and H_5. The almost equal NOE values observed on both inner CD protons when H_o was irradiated, and the fact that the NOE value from H_m to H_3 was larger than that from H_m to H_5 suggest an inclusion geometry with the aromatic H_o proton located at the CD equatorial plane, H_p on the central vertical axis, and H_m at similar distances from H_5 and from H_3. These requirements are only compatible with a fast equilibrium between two inclusion structures (see Figure 2).

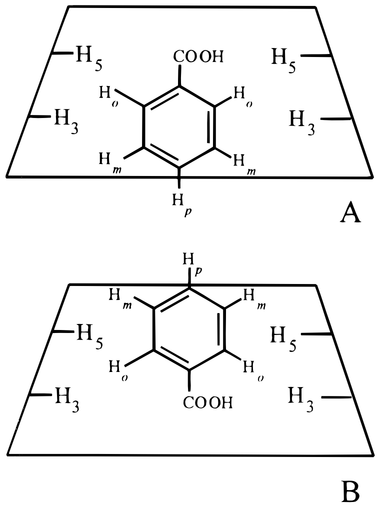

Figure 2. Proposed geometries for the inclusion of benzoic acid into β-CD as deduced from the NOE experiments.

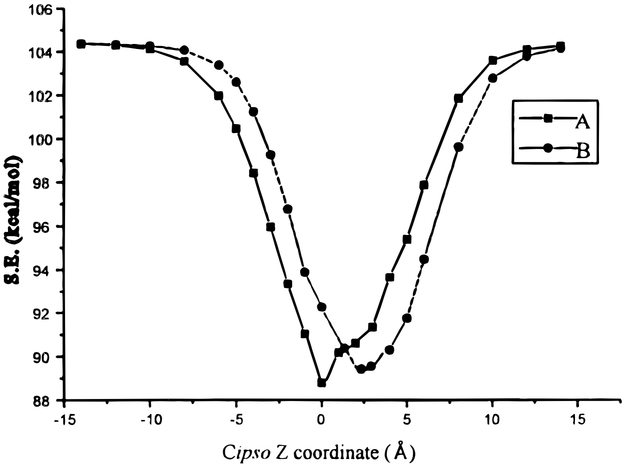

Figure 3. Graphic of the simulation of the inclusion process of benzoic acid into β-CD. Guest position is referred by the C_{ipso} Z coordinate relative to the average plane formed by the glycosydic oxygens (see refs 28−32 for details).

Molecular Mechanics Results. See Experimental Section for more details. Two main relative orientations between the β-CD (as defined by its equatorial plane) and the benzene ring can be considered: (a) parallel, and (b) perpendicular. The first can be discarded just by simple inspection of desktop molecular models since no inclusion complex can be formed, but the second contains two more possibilities depending on which group enters from the larger CD rim: (a) the carboxyl group, (b) the benzene ring. The energy variation associated with the inclusion process was computed for both possibilities (hereinafter called orientation A and B, respectively). The graphic of the steric energy associated with each computed geometry (Figure 3) gave smooth curves for each simulation. Each energy minimum was fully optimized without imposing any restraint. Orientations A and B were computed to be almost isoenergetic (A is only 0.27 kcal/mol more stable than B). The MM2-computed structures for both energy minima are shown in Figure 4. Both computed energy minima present the aromatic H_o protons between inner CD protons, and the H_p proton is always on the central axis of the complex. Moreover, while H_m protons are below H_3 protons in orientation A, they are above H_5 protons in orientation B, in agreement

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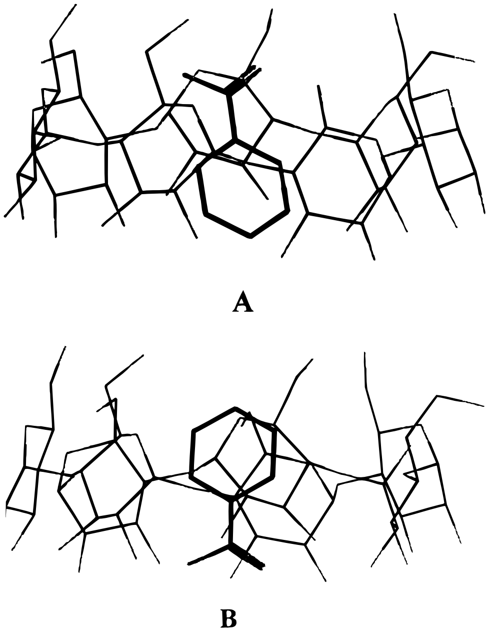

Figure 4. Structures of the energy minima obtained by the MM2 calculations for the β-CD/benzoic acid inclusion complex. Hydrogen atoms have been removed for clarity. (A) Orientation A. (B) Orientation B.

with the geometry derived from the NOE experiments (*vide supra*).

Discussion

The low association constant determined experimentally (about 48 M^{-1}) for the β-CD/benzoic acid complex indicates a very fast equilibrium between both complexed and isolated molecules. This value is in contrast with the association constants published for the complex with α-CD (565 ± 44 M^{-1} at pH 1.5,[21] and 751 ± 1[22]) and for the very same complex (604 M^{-1} in 0.1 M aqueous HCl[17a]). This last association constant was determined using the Benesi–Hildebrand method which requires the use of only those samples where the concentration of one of the components of the complex is much larger than the other. Moreover, usually one single proton is used in these studies giving rise to different constants depending on the proton studied. Our computer program[19] uses all the protons in the complex and requires concentrations of one component to be only larger than those for the other. According to this small association constant, the experimentally determined geometry based on NOE experiments may thus be an average geometry.

The agreement between the geometry found experimentally and the computed structure based on MM2 force field appears to be good. However, while MM calculations give just one single energy minimum for each orientation studied, the reality may be different. Some questions arise: are computed energy minima in agreement with the experimental geometry? Is any other structure near these energy minima in a better agreement with the experimental data? Moreover, are energy minima good representatives for the average guest geometry tumbling inside the host?

Table 3. Effective Distance Ratios between Different Pairs of Protons as Computed for Geometries Near the Energy Minimum in Orientations A and B, as Well as the Root-Mean-Square (rms) Values for Their Comparisons with the Ratios between Corresponding Experimental NOE Values

		$R_{k,j}/R_{i,j}$				
orientation	guest position (C_{ipso} *Z* coord)	$R_{3,o}/R_{5,o}$	$R_{3,m}/R_{5,m}$	$R_{o,3}/R_{m,3}$	$R_{o,5}/R_{m,5}$	rms[a]
A	−2	0.668	0.690	0.659	0.681	0.242
	−1	0.801	0.665	0.763	0.634	0.186
	0	1.054	0.666	0.964	0.609	**0.143**
	1	1.343	0.738	1.259	0.692	0.255
	2	1.627	0.917	1.519	0.856	0.434
	$(NOE_{i,j}/NOE_{k,j})^{1/6}$	0.993	0.906	0.935	0.751	
B	0	0.518	1.107	0.979	1.164	0.498
	1	0.728	1.232	0.837	1.417	0.397
	2	0.941	1.530	0.685	1.114	**0.383**
	3	1.187	1.639	0.623	0.861	0.414
	4	1.505	1.547	0.663	0.682	0.599

[a] Root-mean-square value for the deviation with the experimental set of NOE ratios (central entry).

Although the only qualitative observation of the computed structure gives an affirmative answer to these questions, a quantitative comparison was carried out based on the dependence of experimental NOE values with the inverse of the sixth power of the distance between saturated and observed protons.[23] Based on this relationship, the ratio $(NOE_{i,j}/NOE_{k,j})^{1/6}$ should be equal to $R_{k,j}/R_{i,j}$. One more point should be considered: due to the symmetries of guest and host, we are dealing with 7 or 14 of such pair distances simultaneously (7 inner equivalent CD protons and 1 or 2 equivalent guest protons).

The approximation of Bendall ($1/R^6_{eff} = 1/n\ \Sigma\ R^{-6}{}_i$),[24] originally deduced to consider the contributions from the CHXY rotamers, was used to account for the contribution of each proton considered. It computes the effective distance (R_{eff}) at which two determinate protons are found, and it is fully justified due to a much faster internal rotation of the guest as compared with the reorientation of the complex.

Table 3 contains the comparisons between the experimental NOE ratios and the effective distance ratios for different pairs of protons corresponding to computed geometries at ±2 Å from the energy minimum of orientations A and B. Those structures corresponding to the computed energy minima give rise to the smallest deviation (lower rms). Interestingly, orientation A presents the smallest rms value, while the energy minimum for orientation B presents a 2.5 times larger deviation. The geometrical and energetic comparisons produced coincident results.

The complex geometry deduced in this work is in agreement with that recently proposed for the β-CD/benzoate anion complex.[17b] In this case, only one orientation for the guest is proposed (orientation A) according to the 2D-ROESY experiments. Probably, the negative charge over the carboxylate anion is much better stabilized by the primary hydroxyl groups than by the secondary.

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Although comparison with structures for substituted benzenes can be made, care has to be taken due to the competition of functional groups (bimodal inclusions). The geometrical results obtained in this work qualitatively agree with those already published on related complexes (like the X-ray structure for the β-CD/4-*tert*-butylbenzoic acid complex[25]).

Conclusions

The inclusion complex of benzoic acid and β-CD has been studied. A 1:1 stoichiometry and an association constant of 48.5 M^{-1} were determined based on induced chemical shifts considering all possible protons presenting chemical shifts.[26] This low constant indicates a very fast equilibrium between complexed and free substrates which, in turn, results in an averaged inclusion geometry. The reasonable agreement between the experimentally determined distance ratios (based on NOE values) and the computed geometry for the inclusion (carboxylic group pointing toward the smaller rim) is remarkable. The molecular mechanics-derived structure appears to be a very reasonable representative of the average geometry for the complex in solution.

Experimental Section

β-CD, benzoic acid, and D_2O were purchased from Aldrich, Panreac, and Merck, respectively. Solutions 1.63×10^{-2} M of β-CD and benzoic acid in D_2O were prepared with the help of sonication. Six different samples were prepared by mixing variable volumes of both solutions to obtain host/guest ratios from 3.5 to 0.2.

The NMR spectra were obtained on a Bruker AMX-400 machine at 300 K. Cross-relaxation was achieved using a low-power off-resonance continuous-wave irradiation (2.5 kHz) as a mixing time during 700 ms. 1D-ROESY spectra were acquired with 1024 scans using a relaxation period of 1 s.

Computations were performed in a VAX-6400 computer at the Computing Centre of the UAB. The inclusion of benzoic acid[27] into β-CD was simulated using an already published method.[28−32] Although there is an MM3 force field,[33] Allinger's old MM2(85) force field[34] was used throughout as it offers excellent agreements with experimental data. Solvent was not considered neither implicitly nor explicitly. After properly orienting host and guest, the inclusion process was simulated by successively changing the Z coordinate of the guest atoms.[28−32] The position of the guest relative to the host is referred to the Z coordinate of one reference atom (the C_{ipso}).

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